IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of)
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Giorgio Pelicci et al.)
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Serial No. Not yet assigned)
[Int. Appl. No. PCT/GB00/01079])
)
Filed: March 22, 2000)
)
For: "Materials and Methods)
Relating to Modulation)
of p66 Expression")

PRELIMINARY AMENDMENT

The present application is based on International Application PCT/GB00.01079. Before calculation of the filing fee, please amend the above-referenced patent application as follows:

In the specification:

At page 1, line 3, please insert the following priority claim:

-- This application claims is a §371 filing of International Application PCT/GB00/01079 filed March 22, 2000 which in turn claims priority from GB Application No. 9906515.3, filed March 22, 1999.--

At page 55, on a separate sheet, please insert the following abstract of the disclosure:

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ABSTRACT

It has been determined that i) $p66^{shc}$ is serine phosphorylated upon UV treatment or oxidative damage: ii) the serine-phosphorylation of p^{66} by oxidative signals is mediated by Erk1 and p38, as shown in vivo and in vitro; iii) ablation of $p66^{shc}$ expression by homologous recombination enhances resistance to oxidative damage both in vitro and in vivo; iv)

a serine-phosphorylation defective mutant of $p66^{shc}$ is unable to restore a normal stress response in $p66^{shc}$ targeted cells; v) mice carrying the $p66^{shc}$ targeted mutation have prolonged lifespan.--

In the claims:

2. (Amended) A nucleic acid molecule according to claim 1 wherein the serine residue is selected from the group consisting of S17, S19, S20, S26, S28, S36, S38, S40, S41, S54, S60, S66, S80 and S120.

3. (Amended) A nucleic acid molecule according to claim 1 wherein the serine residue is selected from the group consisting of S28, S36 and S54.

4. (Amended) A nucleic acid molecule according to claim 1 wherein the serine residue is S36 and is replaced by alanine ($p66^{shc}$S36A)

5. (Amended) A polypeptide encoded by a nucleic acid molecule according to claim 1.

6. (Amended) A replicable vector comprising nucleic acid according to claim 1 operably linked to control sequences to directs its expression.

14. (Amended) A method according to claim 12 wherein said step of disrupting the $p66^{shc}$ pathway causes a mutant $p66^{shc}$ polypeptide to be expressed such that at least one serine residue present in the wild type $p66^{shc}$ is absent or replaced by a different amino acid residue.

15. (Amended) A method according to claim 14 wherein said

serine residue is S36 and is replaced by alanine.

17. (Amended) A method according to claim 12 wherein said disruption effects the ability of a serine/threonine kinase, p38 or MAPK to phosphorylate $p66^{shc}$.

22. (Amended) A method for increasing cellular resistance to oxidative stess comprising administration of an effective amount of an agent which disrupts $p66^{shc}$ or a step in the $p66^{shc}$ signalling pathway in a pharmaceutically acceptable carrier .

23. (Amended) A method as claimed in claim 22 wherein said agent is an antisense oligonucleotide capable of specifically hybridising to $p66^{shc}$ nucleic acid.

24. (Amended) A method according to claim 23 wherein said antisense oligonucleotide is RNA

25.(Amended) A method according to claim 23 wherein the $p66^{shc}$ nucleic acid sequence is shown in Fig. 5.

26. (Amended) A method according to claim 22, wherein said agent is an antibody binding domain capable of specifically binding to a $p66^{shc}$ polypeptide or fragment thereof.

27. (Amended) A method as claimed in claim 22 wherein said agent is administered for the treatment of diseases selected from the group consisting of lung emphysema, myocardial infarction, stroke, premature aging, cell senescence, Parkinson's, Alzheimer, cancers and diabetes.

34. (Amended) A method according to claim 32 wherein said step of determining the amount of a compound of the signalling pathway is an enzyme activity assay.

35. (Amended) A method according claim 32 wherein said candidate compounds include nucleic acid sequences, antibody binding domains, and protein nucleic acids.

Please cancel claims 39, 40 and 41.

REMARKS

The purpose of this preliminary amendment is 1) Insert the appropriate priority claim into the specification; 2) Submit an abstract of the disclosure on a separate sheet; and 3) to eliminate multiply dependent claims.

Favorable consideration leading to prompt allowance of the present application is respectfully requested.

Respectfully submitted,
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Enclosures: Marked up draft of amended claims

2. (Amended) A nucleic acid molecule according to claim 1 wherein the serine residue is selected from the group consisting of S17, S19, S20, S26, S28, S36, S38, S40, S41, S54, S60, S66, S80 [or] and S120.

3. (Amended) A nucleic acid molecule according to claim 1 [or claim 2] wherein the serine residue is selected from the group consisting of S28, S36 and S54.

4. (Amended) A nucleic acid molecule according to [any one of the preceding] claim[s] 1 wherein the serine residue is S36 and is replaced by alanine ($p66^{shc}$S36A)

5. (Amended) A polypeptide encoded by a nucleic acid molecule according to [any one of the preceding] claim[s] 1.

6. (Amended) A replicable vector comprising nucleic acid according to [any one of] claim[s] 1 [to 4] operably linked to control sequences to directs its expression.

14. (Amended) A method according to claim 12 [or claim 13] wherein said step of disrupting the $p66^{shc}$ pathway causes a mutant $p66^{shc}$ polypeptide to be expressed such that at least one serine residue present in the wild type $p66^{shc}$ is absent or replaced by a different amino acid residue.

15. (Amended) A method according to claim 14 wherein said serine residue is S36 and is replaced by alanine.

17. (Amended) A method according to [any one of] claim[s] 12 [to 16] wherein said disruption effects the ability of a serine/threonine kinase, p38 or MAPK to phosphorylate $p66^{shc}$.

22. (Amended) [Use of a substance] A method for increasing cellular resistance to oxidative stess comprising administration of an effective amount of an agent which disrupts $p66^{shc}$ or a step in the $p66^{shc}$ signalling pathway[, in the preparation of a medicament to increase cellular resistance to oxidative stress] in a pharmaceutically acceptable carrier .

23. (Amended) [Use of] A method as claimed in claim 22 wherein said agent is an antisense oligonucleotide capable of specifically hybridising to $p66^{shc}$ nucleic acid [in the preparation of a medicament for increasing resistance in cells to oxidative stress].

24. (Amended)[Use] A method according to claim 23 wherein said antisense oligonucleotide is RNA

25.(Amended) [Use]A method according to claim 23 [or claim 24] wherein the $p66^{shc}$ nucleic acid sequence is shown in Fig. 5.

26. (Amended) [Use of] A method according to claim 22, wherein said agent is an antibody binding domain capable of specifically binding to a $p66^{shc}$ polypeptide or fragment thereof [in the preparation of a medicament for increasing resistance in cells to oxidative stress].

27. (Amended)[Use according to any one of] A method as claimed in claim[s] 22 [to 26] wherein said agent is administered [the medicament is] for the treatment of diseases [including] selected from the group consisting of lung emphysema, myocardial infarction, stroke, premature aging, cell senescence, Parkinson's, Alzheimer, cancers and diabetes.

34. (Amended) A method according to claim 32 [or claim 33]

wherein said step of determining the amount of a compound of the signalling pathway is an enzyme activity assay.

35. (Amended) A method according [to any one of] claim[s] 32 [to 34] wherein said candidate compounds include nucleic acid sequences, antibody binding domains, and protein nucleic acids.

MATERIALS AND METHODS RELATING TO MODULATION OF p66 EXPRESSION

Field of the Invention

The present invention relates to materials and methods concerned with the effects of p66 expression. Particularly, but not exclusively, the present invention provides materials and methods relating to observations that p66, and more particularly p66 Shc isoform, is part of a signal transduction pathway that regulates stress response, response to oncogenic signals and lifespan in mammals.

Background of the Invention

The genes that are responsible for the phenomenon of aging in mammals are unknown. Current theories postulate that aging is the consequence of mutations which do not affect fitness of adult individuals, and which have deleterious effects later in life. Circumstantial evidence suggest genes involved in the control of the oxidative stress response are candidate "aging genes". Indeed, accumulation of oxidative damage correlates with aging.

The mammalian SHC locus encodes three isoforms: p52, p46 and p66. They differ by the presence of N-terminal sequences of variable length and share a C-terminal SH2 domain, a central collagen-homology domain (CH1), rich in proline/glycine residues, and an N-terminal phosphotyrosine-binding domain (PTB). The 110 amino acid N-terminal region unique to p66 is also rich in glycine and proline residues (CH2) (Fig.1A). Therefore, $p66^{shc}$ is a splice variant of $p52^{shc}/p46^{shc}$ (Migliaccio E. et al Embo J. 16, 706-716 (1997), a cytoplasmic signal transducer